Proposed Registration Decision PRD2015-09

Autographa californica Nucleopolyhedrovirus FV11

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Overview

Proposed Registration Decision for *Autographa californica* nucleopolyhedrovirus FV11

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of AcMNPV Technical and Loopex, containing the technical grade active ingredient *Autographa californica* nucleopolyhedrovirus FV11, to control cabbage looper larvae in greenhouse cucumber, peppers and tomatoes.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation provides detailed technical information on the human health, environmental and value assessments of AcMNPV Technical and Loopex.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Autographa californica* nucleopolyhedrovirus FV11, the PMRA will consider all comments received from the public in response to this consultation document.[3] The PMRA will then publish a Registration Decision[4] on *Autographa californica* nucleopolyhedrovirus FV11, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation of this consultation document.

What Is *Autographa californica* nucleopolyhedrovirus FV11?

Autographa californica nucleopolyhedrovirus FV11 is the active ingredient in AcMNPV Technical and Loopex. Loopex is a new end-use product that is proposed for use as commercial-class biological insecticide for the control of cabbage looper (*Trichoplusia ni*) larvae on greenhouse grown cucumbers, peppers and tomatos. Loopex is applied as a foliar spray.

Autographa californica nucleopolyhedrovirus FV11 is a naturally occurring baculovirus within the genus *Alphabaculovirus*. It has a host range limited to the larvae of several species of Lepidoptera within the family Noctuidae, including the cabbage looper *Trichoplusia ni*. The baculovirus polyhedral inclusion bodies (PIBs) must be ingested by the larvae in order to be effective. Upon ingestion, the virus replication process is initiated. Virus-infected cells produce non-occluded virus, which spreads infection throughout the host. The virus-infected larva eventually disintegrates and releases new occluded virus particles which may infect other larvae upon ingestion.

Health Considerations

Can Approved Uses of *Autographa californica* nucleopolyhedrovirus FV11 Affect Human Health?

***Autographa californica* nucleopolyhedrovirus FV11 is unlikely to affect your health when Loopex is used according to the label directions.**

People could be exposed to *Autographa californica* nucleopolyhedrovirus FV11 when handling and applying Loopex, and when ingesting treated produce. When assessing health risks, several key factors are considered:

- the microorganism's biological properties (for example, infection cycle);
- reports of any adverse incidents;

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[3] "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

[4] "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

- its potential to cause disease or toxicity as determined in toxicological studies; and
- the level to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns. When other strains of *Autographa californica* nucleopolyhedrovirus or other baculoviruses were tested on laboratory animals and tissue cultures, there were no signs that it caused any significant toxicity or disease. Furthermore, there have been no reported adverse effects despite the natural occurrence and prevalence of baculoviruses in the environment and the limited host range associated with baculoviruses has been well documented.

Residues in Water and Food

Dietary risks from food and water are not of concern.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure that the food Canadians eat is safe.

Residues of *Autographa californica* nucleopolyhedrovirus FV11 on treated food crops, at the time of harvest, are anticipated following foliar applications to agricultural crops. While baculoviruses are not commonly found on food crops, they are abundant in nature; however, no adverse effects from dietary exposure have been attributed to natural populations of *Autographa californica* nucleopolyhedrovirus. Moreover, no adverse effects have been reported in acute oral toxicity and tissue culture studies with other strains of *Autographa californica* nucleopolyhedrovirus or with other studied baculoviruses. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are considered to be negligible and not of concern. Therefore, the Pest Management Regulatory Agency (PMRA) has determined that specification of an MRL under the *Pest Control Products Act* is not required for *Autographa californica* nucleopolyhedrovirus FV11.

Risks in Residential and Other Non-Occupational Environments

Estimated risk for non-occupational exposure is not of concern.

Loopex is proposed for use on greenhouse grown agricultural crops. Consequently, it is unlikely that adults, youths and toddlers will be exposed to *Autographa californica* nucleopolyhedrovirus FV11. Even in the event of exposure, risk to the general population is not a concern since there were no signs of disease or toxicity noted in toxicological studies with other strains of *Autographa californica* nucleopolyhedrovirus (AcMNPV) or other baculoviruses.

Occupational Risks From Handling Loopex

Occupational risks are not of concern when Loopex is used according to label directions, which include protective measures.

Workers handling Loopex can come into direct contact with *Autographa californica* nucleopolyhedrovirus FV11 on the skin, in the eyes or by inhalation. For this reason, the product label will specify that workers exposed to the end-use product must wear a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles and a NIOSH approved mist filtering mask or respirator with any N-95, P-95 or R-95 filter. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where Loopex has been applied until mists have settled.

For the bystander, exposure is expected to be much less than that of handlers and mixer/loaders and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When *Autographa californica* nucleopolyhedrovirus FV11 Is Introduced Into the Environment?

Environmental risks are not of concern.

Autographa californica nucleopolyhedrovirus FV11 is a baculovirus that specifically infects lepidoteran insects.

Loopex is proposed for use as an insecticide to control cabbage looper larvae in greenhouse grown cucumbers peppers and tomatoes and is not intended for aquatic applications. Exposure to outdoor terrestrial and aquatic environments is expected to be minimal.

Acceptable scientific rationales were used to determine that no significant adverse effects to non-target organisms are expected.

Value Considerations

What Is the Value of Loopex?

Loopex controls cabbage looper larvae in greenhouse tomato, cucumber and pepper.

Foliar applications of Loopex control cabbage looper larvae on listed greenhouse crops. Applications should target small larvae. Applications may be repeated every 7-14 days as long as monitoring indicates they are necessary.

Resistance to *Autographa californica* nucleopolyhedrovirus FV11 in cabbage looper has not been reported. *Autographa californica* nucleopolyhedrovirus FV11 represents a new mode of action for use against cabbage looper larvae. Based on the demonstrated efficacy of the product and its compatibility with *Bacillus thuringiensis* (Bt) treatment, chemical insecticides and beneficial insect species, Loopex could be a valuable part of an integrated pest management program on greenhouse tomato, cucumber and pepper. Registration in Canada would address three priorities listed in the Canadian Grower Priority Database.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Loopex to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

In individuals exposed repeatedly to potentially large quantities of Loopex, respiratory and dermal sensitivity may possibly develop. All microorganisms, including *Autographa californica* nucleopolyhedrovirus FV11, contain substances that are potential sensitizers. Therefore, anyone handling or applying Loopex must wear appropriate personal protective equipment including a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles and a NIOSH approved mist filtering mask or respirator with any N-95, P-95 or R-95 filter. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where Loopex has been applied until mists have settled.

Environment

The end-use product label will include environmental precaution statements that prevent the contamination of aquatic systems from the use of Loopex.

Next Steps

Before making a final registration decision on *Autographa californica* nucleopolyhedrovirus FV11, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Autographa californica* nucleopolyhedrovirus FV11 (based on the Science Evaluation of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Autographa californica Nucleopolyhedrovirus FV11

1.0 The Active Substance, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active ingredient	Polyhedral inclusion bodies (PIBs)* of *Autographa californica* nucleopolyhedrovirus FV11
Function	Biological Insecticide –To control cabbage looper larvae (*Trichoplusia ni*) on greenhouse grown cucumbers, peppers and tomatoes
Binomial name	*Autographa californica* nucleopolhedrovirus FV11
Taxonomic designation	
Superkingdom	Viruses
Family	Baculoviridae
Genus	Alphabaculovirus
Species	*Autographa californica* nucleopolyhedrovirus
Strain	FV11 (Fraser Valley #11)
Patent Status information	None.
Nominal purity of active	Technical Grade Active Ingredient (TGAI): minimum of 1×10^9 PIBs/mL End-Use Product (EP): minimum of 5×10^8 PIBs/mL
Identity of relevant impurities of toxicological, environmental and/or significance.	The TGAI does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards.

*PIBs are also referred to as occlusion bodies (OBs)

1.2 Physical and Chemical Properties of the Technical Product and the End-Use Products

End-use Product – Loopex and AcNMPV Technical

Property	Result
Colour	Light-medium brown
Physical State	Liquid, flowable
Odour	Undetectable
Miscibility	n/a; not emulsifiable
Corrosion Characteristics	Non-corrosive
pH	6.0–7.0
Viscosity	12.64 cSt at 20°C and 5.86 cSt at 40°C
Density	1.2 g/mL

1.3 Directions for Use

Foliar applications may be made within the rate range of 2.5×10^{10}- 1×10^{11} PIB/ha. Applications should target small larvae and be applied in a spray volume of 400 L/ha. Repeat applications may be made every 7-14 days, if monitoring indicates that they are necessary.

1.4 Mode of Action

The mode of action associated with *Autographa californica* nucleopolyhedrovirus FV11 is infection leading to larval death. Larval infection begins with ingestion of viral OBs which consist of occlusion derived virions (ODVs) embedded in a proteinaceous matrix. In the alkaline environment of the larval midgut, the protein matrix is dissolved. The released ODVs infect the midgut epithelial cells in which progeny nucleocapsids are produced and bud from the cellular plasma membrane (budded virus or BV). Budded viruses initiate secondary infections in other cells and tissues. At late stages of infection, proteins involved in forming OBs are produced and enclose the virus particles leading to hypertrophy of the infected cells and tissues. As virus replication proceeds, the cells lyse and release progeny OBs into the environment for subsequent ingestion and infection of new hosts.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganisms

Autographa californica nucleopolyhedrovirus FV11 can be identified at the strain level using a combination of deoxyribonucleic acid (DNA) sequencing, gene parity analysis and amino acid sequencing of the lef8 and pif2 open reading frames. Strain FV11 can be distinguished from other strains by polymerase chain reaction (PCR) using specific primer sets and by restriction endonuclease (REN) pattern analysis.

2.2 Methods for Establishment of Purity of Seed Stock

The production strain is maintained as a master seed stock that is stored at -18°C in the form of either unpurified virus in infected *Trichoplusia ni* larval cadavers or as purified occlusion bodies. To ensure consistency, DNA from the original seed stock (produced in 2006), as well as from newly produced seed stock, working stock and the formulated product are subjected to PCR analysis using a specific primer set that was designed to distinguish sequence variations commonly detected in *Autographa californica* nucleopolyhedrovirus variants.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantees of the technical product and the end use product are expressed in units of PIBs/mL. Representative data on five batches of end use product were submitted. Representative data included PIB counts and relative potency results against a reference standard with a known virus concentration. Methods for determining the concentration of PIBs and relative potency were adequately described.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

As noted above, appropriate methods are available to enumerate PIBs and to distinguish this microbial pest control agent (MPCA) from other strains of *Autographa californica* nucleopolyhedrovirus and other closely related baculoviruses. However, no methods are required to quantify viable or non-viable residues of *Autographa californica* nucleopolyhedrovirus FV11 in food as it is a ubiquitous microorganism in nature and has been isolated from a wide variety of environments.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during the manufacture of AcMNPV Technical and Loopex are acceptable. These procedures include good hygienic practices for the maintenance, sanitation and cleaning of all laboratories, decontamination of eggs and daily monitoring of the cabbage looper colony.

The absence of human pathogens and below-threshold levels of contaminating microorganisms were shown in the microbial screening of production batches using microbe-specific screening methods for detecting and enumerating microbial contaminants of concern. Release standards for microbial contaminants comply with those permitted by the PMRA and are adequate to ensure that the end-use product does not contain unacceptable levels of human and animal disease-causing microorganisms.

Although not a part of the routine quality assurance protocol, a mouse intraperitoneal assay was also conducted on five batches of *Autographa californica* nucleopolyhedrovirus FV11. No treatment-related adverse effects were noted.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

The applicant will be conducting a storage stability study testing the stability of Loopex over the course of one year when stored at 5°C. In addition to assessing the stability of the end use product, the stability of the container, physical properties and microbial contamination will be monitored. Until complete storage stability data are available, the labels for AcMNPV Technical and Loopex must indicate a maximum storage period of six months at 4°C or less.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

Scientific waiver rationales were submitted to address all human health and safety requirements for AcMNPV Technical and Loopex (see Appendix I) as no human health and safety studies were provided specifically for *Autographa californica* nucleopolyhedrovirus FV11. A general scientific waiver rationale was provided to address exposure through various routes. In addition, studies on other strains of *Autographa californica* nucleopolyhedrovirus or other baculoviruses were provided to address specific routes of exposure.

Baculoviruses are naturally occurring and ubiquitous in the environment. *Autographa californica* nucleopolyhedrovirus occlusion body (OB) counts on random samples of cabbage taken from store shelves or collected from the field vary between 3.1×10^5 OBs/cm^2 to 1.1×10^7 OBs/cm^2. Based on these numbers, it is estimated that a typical serving of cole slaw contains an average of 1×10^8 OBs. Despite the close interaction between baculoviruses and humans, no reports of adverse effects have been noted.

The host range associated with baculoviruses is limited to arthropods. Baculoviruses are considered to be highly host specific, infecting only one or a few closely related species within the same order from which they were initially isolated. The active ingredient in Loopex is in the form of OBs. The ODVs are released only when the proteinaceous matrix is dissolved in the alkaline environment (pH 8–11) of the larval midgut. Organisms in which the digestive tract or other ports of entry are not alkaline would not be susceptible to viral OBs. Although non-occluded forms of baculoviruses (i.e. alkaline-liberated virus, pre-occluded virus or BV) can enter cells from non-permissive hosts, the viral DNA does not reach the nuclei in an expressible form. As a result, no cytopathic effects, evidence of virus replication or viral gene expression have been noted even at high multiplicities of infection.

In addition to the lack of infection and replication in vertebrate cells, no evidence of baculovirus-induced cytogenic, carcinogenic, mutagenic or teratogenic effects has been observed.

The results of numerous safety studies with baculoviruses have been extensively reviewed. Baculoviruses have been administered to a wide range of vertebrates, including mammals, at doses many times higher than that acquired in the field through all possible routes of exposure (for example, oral, inhalation, intravenous, intracerebral, intramuscular and dermal). There were no instances of toxicity, allergic response or evidence of pathogenicity. In long-term oral and parenteral studies on rats, no baculovirus-related deaths or neoplasia were observed.

Baculoviruses are also classified by the European Union as being low risk biocontrol agents. Laboratories in which baculoviruses are studied are rated as BioSafety Level 1 (BSL-1) which is reserved for the lowest risk microbial organisms that are not known to consistently cause disease in healthy adults. Work is typically conducted on open benches using standard microbiological practices. Special containment equipment or facility design is not required.

Summary of Acute Oral Infectivity and Toxicity Studies

The potential effects of AcAaIT, a recombinant *Autographa californica* nucleopolyhedrovirus modified to express an insect-selective neurotoxin isolated from the scorpion *Androctonus australis*, wild-type *Autographa californica* nucleopolyhedrovirus and wild-type *Spodoptera littoralis* NPV (SlNPV) were studied in albino rats. Rats (3/sex/virus) were orally administered AcAaIT, *Autographa californica* nucleopolyhedrovirus or SlNPV at a dose of 1×10^8 OBs/rat and then observed for 21 days. There were no mortalities and all animals appeared healthy and gained weight over the course of the study. No consistent differences in blood composition or blood chemistry were noted between control and treatment groups. Tissues from the stomach, intestine, liver, kidney, brain, spleen and lungs were examined for histopathological effects and only slight changes were noted. It was concluded that there were no overt signs of toxicity amongst rats orally exposed to AcAaIT, *Autographa californica* nucleopolyhedrovirus or SlNPV. The oral LD_{50} in rats was greater than 1×10^8 OBs/animal. Any apparent toxicity was attributed to a general response to a foreign body.

SPF-Wistar rats (20/sex) were orally administered *Autographa californica* nucleopolyhedrovirus in a single dose of 5×10^9 OBs/kg bw. Animals were observed for 21 days post-treatment. Two animals/sex were sacrificed on Days 0, 1, 3, 7 and 14 and three animals/sex were sacrificed on Day 21. One male rat in the *Autographa californica* nucleopolyhedrovirus-treated group died three days after administration. Upon necropsy, however, this death was attributed to rectal rupture likely due to an injury incurred during temperature measurement. No changes in behaviour were noted and mean body weights increased over the course of the study. No significant differences in body weights or food consumption were observed between treatment and control groups. An increase in body temperature was noted amongst male rats treated with *Autographa californica* nucleopolyhedrovirus on Days 0, 10 and Day 18 but was not considered to be toxicologically relevant as the difference was slight and not consistently observed. The hematology and clinical chemistry parameters remained within the range of normal biological variation. There were no statistically significant changes in relative organ weights. Two males in the treatment group presented with bilateral hydronephrosis but microscopic examination of the organs did not indicate any morphogically recognizable damage.

In another study, gypsy moth (*Lymantria dispar*) nucleopolyhedrovirus (LdNPV) was orally administered to Sprague-Dawley rats (20/sex) at a dose of 4×10^{10} OBs/animal. The study was 35 days in duration. Two animals/sex were sacrificed on Day 0 (immediately after dosing) and on Days 1, 3, 7 and 14. In addition, three animals/sex were sacrificed on Days 21 and 35. There were no mortalities and all animals gained weight normally. There were no differences between treatment and control groups in body weights or food consumption. The body temperature data indicates a few instances of possible fever. These instances, however, were recorded with equal frequency amongst the control group and were recorded late in the observation period suggesting

that they were not treatment-related. No significant differences in blood chemistry were noted and all haematological values were within normal ranges. At study termination, the necropsies on the remaining animals revealed no deviations from normal and no differences in organ weights were observed with the exception of increased pituitary weight in treated females but this was attributed to coincidental early manifestation of pituitary tumours normally seen in rats of this strain. Histopathological examination of various tissues and lymph nodes did not reveal any lesions attributable to the virus.

In the final oral study, LdNPV was administered to Sprague-Dawley rats (20/sex) at a dose equivalent to a 100-acre dosage administered to a 70 kg man (i.e. a 1-acre dose was defined as 2×10^{11} OBs). Following treatment, the animals were observed for 30 days. There were no mortalities or abnormal behaviours. No pathological differences were noted in the organs of treatment animals as compared to those of the control animals.

Summary of Acute Pulmonary Infectivity and Toxicity Studies

In an inhalation toxicity study, Sprague-Dawley rats were exposed to occlusion bodies (OBs) of LdNPV via a nose-only inhalation exposure chamber. During the exposure period, the rats were exposed to a dosage of virus used to treat 1 acre (4.9×10^{9}–1.02×10^{11} OBs). Following treatment, the rats were observed for 14 days. Two animals (1/sex) were sacrificed at 0, 1, 2, 4 and 24 hours and at 3 and 7 days after exposure. Four animals (2/sex) were euthanized on Day 14. There were no mortalities, signs of toxicity or abnormal behaviour. No treatment-related abnormalities were noted at necropsy. With the exception of one female rat that was scheduled for sacrifice at 24 hours after exposure, all animals either retained their weight (animals sacrificed <24 hours after exposure) or gained weight (animals sacrificed ≥24 hours after exposure).

In a second study, young adult rhesus monkeys (5/sex) were administered *Heliothis* nucleopolyhedrovirus. At the beginning of the study, four animals (2/sex) received a single 1.2×10^{8} OBs/kg bw dose of virus (equivalent to a 70 kg man receiving virus that would be applied to 1/25 acre). The remaining animals (3/sex) received 26 weekly doses of 1.2×10^{8} OBs/kg bw. One monkey/sex that received the single dose was sacrificed on Day 33. The remaining animals were sacrificed at the end of the study. All animals appeared to be in good health for the duration of the study and normal weight gains and body temperatures were noted. No deleterious effects attributable to the virus were observed upon necropsy. Relative organ weights were within normal ranges. Except for isolated incidents, there were no differences in haematological values between untreated and control animals; by Day 197, values for all animals were within normal ranges. Similarly, there were no differences in blood chemistry. No abnormalities were observed in the histopathological examination of tissues from monkeys sacrificed on Day 33. A greater frequency of lymphoid hyperplasia was observed in the virus-exposed monkeys that had received weekly dosing of virus. Examination of the lymph nodes, however, indicates that these animals were negative for presence of infectious virus or viral antibodies. To detect infectious virus, samples of blood drawn prior to exposure and on Days 7, 28, 120 and 182 as well as samples of mesenteric and bronchial lymph nodes were incorporated into a larval diet and fed to neonatal bollworms. No larval deaths occurred indicating the absence of infectious virus in these samples.

Summary of the Intravenous Infectivity Study

In an acute intravenous study, groups of young adult rats (4/sex) were intravenously injected with 0.25 mL of *Neodiption abietis* nucleopolyhedrovirus (NeabNPV) containing a target dose of 10^7 OBs. An equal number of animals were treated with 0.25 mL of inactivated product in the same manner. The rats were observed for 23 days. There were no mortalities and no clinical signs of toxicity at any point of the study. All animals appeared normal in general condition, demeanour and movement. There were no statistically significant differences in body weight change among the groups throughout the study period. There were no gross necropsy findings for any of the animals in the study.

Summary of Acute Dermal Toxicity Studies

Albino guinea pigs (5/sex) were exposed to 0.1 mL doses of polyhedral of *Autographa californica* nucleopolyhedrovirus applied to two abraded and two intact skin areas of their shaved backs. Each 0.1 mL dose contained 4.35×10^6 OBs. An additional 5 guinea pigs/sex were similarly administered alkaline-liberated virus with each dose containing virus particles derived from 4.35×10^6 OBs. The animals were then observed for 14 days. There were no mortalities. With the exception of one female that produced soft feces on Day 6, all other animals appeared normal throughout the study. All dermal sites were negative at all times. No significant differences in body temperature, body weight, food consumption or mean organ weights were observed between the treatment and control groups. At necropsy, a gross observation of the animals revealed no treatment-related lesions.

In another acute dermal toxicity study, young adult New Zealand white rabbits (5/sex) were dermally exposed to a single dose of NeabNPV (4.0×10^9 OBs/mL) at a dose of 2 g/kg bw to an area of approximately 12 × 14 cm of the rabbit's trunk. The exposure period was 24 hours after which the animals were observed for 15 days. There were no mortalities and no signs of toxicity reported in any of the rabbits. All body weights remained within 20% of the mean of each sex on each study day. In this study, NeabNPV was not toxic to rabbits via the dermal route of exposure.

Summary Dermal Irritation Studies

A primary skin irritation study was conducted in which six New Zealand white rabbits were dermally exposed to LdNPV at a dose of 4.0×10^{10} OBs/animal. The exposure sites had been previously clipped and the skin of three rabbits was abraded while the skin of the remaining three animals remained intact. The test substance was left in contact with the skin for 24 hours. At the end of the exposure period, the test sites were cleaned and dermal reactions were scored in accordance with the Federal Hazardous Substances Act (FHSA). Observations of the treated skin at 24 and 72 hours after test substance administration revealed no evidence of irritation in either intact or abraded skin.

No edema was noted in any of the animals at 24 or 72 hours. Body temperatures were within normal temperature range for rabbits except for one animal whose temperature was slightly depressed through the study; this was not considered to be treatment-related.

In a second study, six New Zealand white rabbits were exposed to *Autographa californica* nucleopolyhedrovirus via a single topical application of 2.2×10^7 OBs/animal. The skin of each rabbit had been clipped and abraded prior to application. Observations for signs of dermal irritation were made at 24 hours and 72 hours after exposure. The dermal reactions were graded according to the Draize method. No signs of dermal irritation were observed in any of the animals.

In another acute dermal irritation study, Granupom SC, a formulation containing *Cydia pomonella* granulovirus (CpGV), was applied to the shaved skin (3×5 cm^2) of three albino rabbits. The test substance was held in place for 4 hours after which the test substance was removed with water. A shaved area close to the exposure area of each animal served as an untreated control. The animals were observed for 10 days. Skin irritation was scored (according to the Draize method) 1 hour after test substance removal and then daily for the remainder of the study. None of the animals exhibited any signs of toxicity. All animals gained weight and appeared normal. Erythema and edema were not noted in any of the animals at any of the observation timepoints.

The waiver rationale submitted to address the potential dermal toxicity of the formulant is also cited to address its potential for dermal irritation.

Summary of Sensitization Study

A sensitization study was conducted with Lecontvirus, an end use product formulation of *Neodiprion lecontei* nucleopolyhedrovirus (NeleNPV) containing 2.0×10^{10} OBs/g. The test substance was prepared by suspending Lecontvirus in a minimum amount of phosphate buffered saline (PBS). The hair of 11 young adult guinea pigs was shaved in parallel bands down the left and right sides of the body. The first sensitizing injections were made at the anterior end of each shaved strip. For each animal, the test substance was administered on the left side of the body and the positive control, 25 µg/mL dinitrochlorobenzene (DNCB), was injected on the right. The initial intradermal injection was 0.05 mL for both the test substance and the positive control. The dose was increased to 0.1 mL for the remaining injections which were administered on alternate days for three weeks. A new location was chosen along the strip for each subsequent injection. There were a total of 10 injection sites for each inoculum. Two weeks after the last injections, the animals were challenged with a final injection of the test substance and the positive control. Dermal reactions were recorded at 24 hours and 48 hours post injection for each animal according to the Draize method. The dimensions of the cutaneous flare and double skin thickness were measured. The double skin thickness values were translated to Draize edema scores. Although the dermal reaction scores for Lecontvirus were consistently higher than for the positive control, the reaction remained relatively constant despite subsequent injections. The dermal reaction scores for DNCB, however, were characterized by a progressive increase as the study continued.

Summary of Tissue Culture Study

A study was conducted on the effects of alkali-liberated *Choristoneura fumiferana* nucleopolyhedrovirus (CfNPV) on the metabolic processes of vertebrate cells. Confluent

monolayers of mouse epithelial cells (L cells), chick embryo fibroblasts (CEF), trout cells (RTG-2) and fat headed minnow cells (FHM) were inoculated with either CfNPV or a negative control. The virus was allowed to adsorb for two hours before radioactive uridine, thymidine or leucine were added to the cultures at one, two and three days after infection. At 2-hour intervals thereafter, the cells were processed and the synthesis of ribonucleic acid (RNA), DNA or protein was determined based on the level of incorporated radioactivity. Synthesis of RNA, DNA and protein increased linearly with time and there was no difference between treated and negative control cells. The virus had no effect on cellular metabolic processes at either the temperature optimal for the cell lines tested or for the replication of CfNPV in Cf cells. There was no evidence that CfNPV replicated or partially expressed in non-target vertebrate cells.

A similar study using NeleNPV also failed to affect protein and RNA synthesis in these same cell lines. Furthermore, cell lysates were subjected to gel electrophoresis followed by autoradiography of protein or fluorography of RNA. The pattern of protein and RNA bands was not altered by inoculation with virus.

In a more extensive study, 35 nontarget cell lines (23 of human origin and 12 of non-human vertebrate origin) were exposed to *Autographa californica* nucleopolyhedrovirus budded virus, occlusion derived virus, preoccluded virus or hemolymph-derived virus. The virus and cells were incubated together at two different temperatures (28 or 37°C) for four different lengths of time (16, 40, 64 or 168 hours) and the cells were assayed for the presence of virus by a peroxidase-antiperoxidase detection method. Although virus uptake appeared to be quite common, as confirmed by electron micrographs in which nucleocapsids were present in the cytoplasm and the vacuoles, there was no evidence of virus particles in the nucleus nor was there evidence of active viral gene expression or viral replication.

Based on the results of these studies, it can be concluded that baculoviruses do not infect cells from non-permissive hosts.

A waiver rationale was also submitted and accepted to address the potential toxicity of the formulation ingredients based on their widespread use and/or presence in industrial and consumer products including pharmaceuticals, cosmetics, food and drinks, paints, resins and paper.

3.1.1 Incident Reports Related to Human and Animal Health

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the PMRA website. Incidents were reviewed for *Autographa californica* nucleopolyhedrovirus FV11 as well as other registered baculoviruses (Douglas fir tussock moth nucleopolyhedrovirus, NeleNPV, LdNPV, CpGV strains CMGV4 and M, and NeabNPV). As of 22 August 2014, no incident reports involving these baculoviruses have been reported to the PMRA.

3.1.2 Hazard Analysis

The database submitted in support of registering AcMNPV Technical and Loopex was reviewed from the viewpoint of human health and safety and was determined to be sufficiently complete to permit a decision on registration.

Human health and safety requirements for AcMNPV Technical and Loopex were addressed with scientific waiver rationales based on the lack of reported adverse effects despite the natural occurrence and prevalence of baculoviruses in the environment, the limited host range associated with baculoviruses, the blocks to infection in non-permissive cells and the results of numerous safety studies conducted with baculoviruses on vertebrates and mammals.

Human health and safety studies using other strains of *Autographa californica* nucleopolyhedrovirus and other baculoviruses indicate that *Autographa californica* nucleopolyhedrovirus FV11will likely be of low toxicity via the oral, pulmonary, intravenous and dermal routes of exposure and will not be a dermal irritant. These studies also indicate that while virus uptake can occur in non-permissive cells such as those of vertebrates, infection will not occur as there is no viral DNA replication or expression of viral proteins.

No additional dermal toxicity or dermal irritation is expected based on the formulation ingredients present in Loopex.

An eye irritation study was not submitted and was not addressed with a scientific waiver rationale. In the absence of these data, the products are assumed to eye irritants and the signal words "CAUTION – EYE IRRITANT" must appear on the labels for the technical and the end use product.

Although the applicant submitted a study indicating that another baculovirus was not a sensitizer, the signal words "POTENTIAL SENSITIZER" must appear on the labels for the AcMNPV Technical and Loopex as all microorganisms are recognized as being able to produce substances that can elicit allergic reactions after repeated exposures to high concentrations.

Higher tier subchronic and chronic toxicity studies were not required because of the anticipated low acute toxicity of Loopex, and the lack of infectivity, toxicity or pathogenicity when various baculoviruses were administered to test animals via the oral, pulmonary, intravenous, and dermal routes of exposure.

Within the available scientific literature, there are no reports that suggest *Autographa californica* nucleopolyhedrovirus FV11 or other baculoviruses have the potential to cause adverse effects on the endocrine system of animals. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *Autographa californica* nucleopolyhedrovirus FV11.

3.2 Occupational, Residential and Bystander Risk Assessment

3.2.1 Occupational Exposure and Risk

When handled according to the label instructions, the potential for dermal, eye and inhalation exposure for applicators, mixer/loaders, and handlers exists, with the primary exposure route being dermal. Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Autographa californica* nucleopolyhedrovirus FV11 has not been identified as a dermal wound pathogen and does not contain any known toxic secondary metabolites. There is no indication that it could penetrate intact skin of healthy individuals. Furthermore, toxicity testing with various baculoviruses showed no significant signs of toxicity via the oral, pulmonary or dermal routes of exposure. No evidence of skin irritation was noted in the submitted dermal irritation studies conducted with various baculovirus preparations. As an eye irritation study was not submitted, Loopex must be considered an eye irritant.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions, regardless of the outcome of sensitization testing. Therefore, anyone handling or applying Loopex must wear a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles and a NIOSH approved mist filtering mask or respirator with any N-95, P-95 or R-95 filter. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where Loopex has been applied until mists have settled.

Label warnings, restrictions and risk mitigation measures are adequate to protect users of Loopex and no significant occupational risks are anticipated for these products.

3.2.2 Residential and Bystander Exposure and Risk

Adults, youths and toddlers are unlikely to be exposed to *Autographa californica* nucleopolyhedrovirus FV11 as Loopex is to be used in greenhouses only. In the event of exposure, the PMRA does not expect that residential and bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *Autographa californica* nucleopolyhedrovirus FV11 and Loopex. It is also assumed that precautionary label statements will be followed by commercial applicators in the use of Loopex. As well, AcMNPV is a species that is ubiquitous in the environment and the use of Loopex is not expected to cause sustained increases in exposure to bystanders beyond natural levels. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Although the proposed use pattern may result in dietary exposure with possible residues in or on agricultural commodities, dietary risk is expected to be negligible and of no concern for the general population, including infants and children, or animals because various baculoviruses demonstrated no pathogenicity, infectivity or oral toxicity in acute oral toxicity and tissue culture studies. Furthermore, higher tier subchronic and chronic dietary exposure studies were not required because of the anticipated low toxicity and lack of infectivity or pathogenicity associated with the MPCA.

3.3.2 Drinking Water

Health risks are not expected from exposure to this microorganism via drinking water because exposure will be minimal and because there are no anticipated harmful effects for *Autographa californica* nucleopolyhedrovirus FV11 as evidenced by acute oral toxicity testing and tissue culture studies using other baculoviruses. The end use product label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Users are also requested not to allow effluent or runoff from greenhouses containing this product to enter lakes, streams, ponds or other waters. Furthermore, municipal treatment of drinking water is expected to remove the transfer of residues to drinking water. Therefore, potential exposure to *Autographa californica* nucleopolyhedrovirus FV11 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e. no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that the *Autographa californica* nucleopolyhedrovirus FV11 is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and the cumulative effects on infants and children of the MPCA and other registered micro-organisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *Autographa californica* nucleopolyhedrovirus FV11 to human health.

3.3.4 Aggregate Exposure and Risk

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty that no harm will result from aggregate exposure of residues of *Autographa californica* nucleopolyhedrovirus FV11 to the general Canadian population, including infants and children, when the EP is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Furthermore, few adverse effects from exposure to other isolates of *Autographa californica* nucleopolyhedrovirus or other baculoviruses encountered in the environment have been reported. Even if there is an increase in exposure to this active ingredient from the use of Loopex, there should not be any increase in potential human health risk.

3.3.5 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally specified as a maximum residue limit (MRL) under the *Pest Control Products Act* for the purposes of the adulteration provision of the *Food and Drugs Act*. Health Canada specifies science-based MRLs to ensure the food Canadians eat is safe.

Residues of *Autographa californica* nucleopolyhedrovirus FV11 on treated food crops, at the time of harvest, are anticipated following foliar applications to agricultural crops. The PMRA has applied a hazard-based approach for determining whether an MRL is required for this microorganism. No adverse effects from dietary exposure have been attributed to natural populations of *Autographa californica* nucleopolyhedrovirus, and no adverse effects were observed in the acute oral toxicity and tissue culture studies with other strains of *Autographa californica* nucleopolyhedrovirus or other baculoviruses. In addition, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Therefore, the PMRA has determined that specification of an MRL under the *Pest Control Products Act* is not required for *Autographa californica* nucleopolyhedrovirus FV11.

3.4 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity. Besides naturally occurring strains of *Autographa californica* nucleopolyhedrovirus or other baculoviruses in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with *Autographa californica* nucleopolyhedrovirus FV11. No cumulative effects are anticipated if the residues of *Autographa californica* nucleopolyhedrovirus FV11 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Autographa californica nucleopolyhedrovirus FV11 belongs to the genus *Alphabaculovirus* in the family Baculoviridae. Baculoviruses are ubiquitous and persistent in aquatic and terrestrial ecosystems. The host range of baculoviruses is restricted to terrestrial arthropods primarily of the larval stage.

The crystalline structure of the OBs has been shown to assist in the dispersal of the virus by vertebrates. The acidic pH (pH 1 to 7) of the stomach of vertebrates helps to preserve the integrity of the OBs. Excreted OBs, recovered from the digestive tracts of non-host invertebrate and vertebrate animals were found to remain infectious to their insects larval hosts, leading to the suggestion that the consumption of baculovirus-infected larvae by various non-target animals plays a role in the dissemination of OBs.

Baculoviruses are a natural component of the host insect's habitat, and environmental concentrations reported in soil (1.55×10^5 PIBs/cm^3), ground litter (4×10^5 PIBs/cm^3) and tree bark (5×10^6 PIBs/cm^3) can persist for at least one year following natural epizootics of the host. Given that *Autographa californica* nucleopolyhedrovirus is abundant in nature, the greenhouse use of Loopex is not expected to result in significant increases of *Autographa californica* nucleopolyhedrovirus FV11 in terrestrial and aquatic environments.

4.2 Effects on Non-Target Species

PMRA has a four-level tiered approach to environmental testing of microbial pesticides. Tier I studies consist of acute studies on up to seven broad taxonomic groups of non-target organisms exposed to a maximum hazard or Maximum Challenge Concentration (MCC) of the MPCA. The MCC is generally derived from the amount of the MPCA or its toxin expected to be available following application at the maximum recommended label rate multiplied by some safety factor. Tier II studies consist of environmental fate (persistence and dispersal) studies as well as additional acute toxicity testing of MPCAs. Tier III studies consist of chronic toxicity studies, i.e. life cycle studies, as well as definitive toxicity testing, for example, LC_{50}, LD_{50}. Tier IV studies consist of experimental field studies on toxicity and fate, and are required to determine whether adverse effects are realized under actual use conditions.

The type of environmental risk assessment conducted on MPCAs varies depending on the tier level that was triggered during testing. For many MPCAs, Tier I studies are sufficient to conduct environmental risk assessments. Tier I studies are designed to represent "worst-case" scenarios where the exposure conditions greatly exceed the expected environmental concentrations. The absence of adverse effects in Tier I studies are interpreted as minimal risk to the group of non-target organisms. However, higher tiered studies will be triggered if significant adverse effects on non-target organisms are identified in Tier I studies. These studies provide additional information that allows PMRA to refine the environmental risk assessments. In the absence of adequate environmental fate and/or field studies, a screening level risk assessment can be performed to determine if the MPCA is likely to pose a risk to a group of non-target organisms.

The screening level risk assessment uses simple methods, conservative exposure scenarios (for example, direct application at a maximum application rate) and sensitive toxicity endpoints. A risk quotient (RQ) is calculated by dividing the exposure estimate by an appropriate toxicity value (RQ = exposure/toxicity), and the risk quotient is then compared to the level of concern (LOC).

If the screening level risk quotient is below the level of concern, the risk is considered negligible and no further risk characterization is necessary. If the screening level risk quotient is equal to or greater than the level of concern, then a refined risk assessment is performed to further characterize the risk. A refined assessment takes into consideration more realistic exposure scenarios (environmental fate and/or field testing results). Refinements to the risk assessment may continue until the risk is adequately characterized or no further refinements are possible.

4.2.1 Effects on Terrestrial Organisms

Acceptable scientific rationales were submitted to waive Tier I testing requirements for F11 to terrestrial non-target organisms based on extensive literature reviews including results of ecotoxicological testing conducted on various baculoviruses. The rationales were based on the following:

- baculoviruses are not toxic to vertebrate animals (birds and mammals), non-arthropod invertebrates, microorganisms and plants;
- baculoviruses are infectious only to insects of the same order from which they were initially isolated;
- baculoviruses are ubiquitous and persistent in aquatic and terrestrial ecosystems yet there has been no report of negative impact of baculoviruses on ecosystems other than the effect on the target host insect; and
- no evidence of infection, toxicity or mortality was observed following exposure to direct deposit of contaminated material (insects, frass, etc.).

Birds

In a 21-day dietary study to examine the effect of two avian predators of gypsy moth, *Lymantria dispar* nucleopolyhedrovirus infected gypsy moth larvae were fed to three black–capped chickadees *Parsus atricapillus* (70 to 80 larvae at a dose of 2.3×10^9 to 1.7×10^{10} PIBs/bird), and to five house sparrows, *Paser domesticus* (90 to 100 larvae at a dose of 3.0×10^9 to 2.1×10^{10} PIBs/bird). On Day 22, birds were weighed and a necropsy with histopathological examinations was performed. There were no reported differences in body weight change or significant histopathological findings. The authors concluded that *Lymantria dispar* nucleopolyhedrovirus had no short-term adverse effect on the two avian predators of gypsy moth from dietary consumption of infected larvae.

In another study, the short term effects from the aerial application of *Lymantria dispar* nucleopolyhedrovirus at 2.5×10^{12} OBs/hectare were determined on caged quails and wild birds. The quails were divided into 2 groups; a control group (5 males and 5 females) and an *Lymantria dispar* nucleopolyhedrovirus -treated group (7 males and 6 females) that were caged and placed on their respective plots before treatment was initiated. Control plots were left untreated while

Lymantria dispar nucleopolyhedrovirus (LdMNPV)-treated plots were sprayed twice at a rate of 2.5×10^{12} OBs/hectare. The quails were maintained in the plots for the entire treatment period and an additional three weeks following the second application. The wild bird population was evaluated for abundance and species composition in both control and *Lymantria dispar* nucleopolyhedrovirus-treated 10-hectare plots at three time points that extended from before treatment through to 1.5 months after the second treatment. As part of the wild bird census, for a two-month period following *Lymantria dispar* nucleopolyhedrovirus treatment, wild birds were collected on a weekly basis. Necropsy and histopathology examinations were carried out on treated and untreated caged quails and wild birds. To ensure that the birds had ingested *Lymantria dispar* nucleopolyhedrovirus, standard bioassays were carried out on gypsy moth larvae using alimentary tract content of control and *Lymantria dispar* nucleopolyhedrovirus - treated birds. No changes in the wild bird population were observed between pre-spray and post-spray evaluations. No significant differences in organ weights, necropsy and histopathology of organs and tissues were observed for any species between *Lymantria dispar* nucleopolyhedrovirus-treated and control birds. Viable *Lymantria dispar* nucleopolyhedrovirus was recovered from the alimentary tract of both control and *Lymantria dispar* nucleopolyhedrovirus-treated birds; however a higher percentage of birds and a larger amount of virus was ingested in *Lymantria dispar* nucleopolyhedrovirus-treated blocks. The aerial application of *Lymantria dispar* nucleopolyhedrovirus had no short-term effect on birds that fed on NPV contaminated food source. In addition, the viability of alimentary tract collected OBs suggest that the virions remain intact in the bird gastrointestinal tract.

In a third study, chickens and turkeys were tested with the nucleopolyhedrovirus for the Redheaded Pine Sawfly (NeleNPV). The birds were divided into 4 groups of 12 (6 males and 6 females) per species: Group 1 (0.8% saline), Group 2 (Non-infected larvae), Group 3 (NPV infected larvae) and Group 4 (NPV purified polyhedral). Test birds were orally dosed with 1.4×10^6 virus particles per gram of body weight per bird. The birds were culled according to the following schedule: Day 1 (2 birds from each group/species; largest male, smallest female), Day 7 (2 birds from each group/species; smallest male and largest female), Day 14 (2 birds from each group/species; largest male and smallest female) and Day 21 (6 birds from each group/species; 3 males and 3 females). There were no pathological differences detected in any test animals of either species. There was no significant difference between body weight and organ weights. The hematology and clinical parameters which were measured showed no significant differences within the test subjects.

Wild Mammals

In a field study, the response of resident populations of white footed mice, *Peromyscus leucopus*, redbacked voles, *Clethrionomys grapperi*, opossums, *Didelphis marsupialis*, chipmunks, *Tamias striatus* and raccoons, *Procyon lotor*, was evaluated to detect any short-term effects from the aerial application of *Lymantria dispar* nucleopolyhedrovirus (2.5×10^{12} PIBs/ha). The study areas were set up in fifteen 14-hectare plots. Caged mice and opossums were placed in treated and control areas. The animals were maintained in the plots for the entire treatment period and an additional four weeks following second application. Wild mammal populations were evaluated by trapping in control and treated plots over a period of two months overlapping the treatment period. Free-living animals (250) were trapped on a weekly basis starting when the first

Lymantria dispar nucleopolyhedrovirus gypsy moth mortality was observed and for the following two months. Necropsy and histopathology examinations were performed on treated and untreated caged animals. To ensure the animals had ingested *Lymantria dispar* nucleopolyhedrovirus, standard bioassays were carried out on gypsy moth larvae using alimentary tract content of 48% of the control and *Lymantria dispar* nucleopolyhedrovirus-treated areas. The data from 47 caged and 250 free-living mammals showed no significant differences in organ and tissue weights, hematological values or necropsy and histopathological findings between treated and control animals. Viable *Lymantria dispar* nucleopolyhedrovirus was recovered from the alimentary tract of both control and *Lymantria dispar* nucleopolyhedrovirus-exposed mammals, however there was a higher percentage and a larger amount of virus ingested by *Lymantria dispar* nucleopolyhedrovirus-treated blocks. There were no short term adverse effects in animals which either contacted the NPV following application or subsequently fed on NPV-infected gypsy moth larvae or other contaminated sources of food. The aerial application of *Lymantria dispar* nucleopolyhedrovirus had no short term adverse effects on mammals that fed on NPV contaminated food sources. Furthermore, the virus remained viable as it passed through the alimentary tract of small mammals.

Terrestrial arthropods

Bioassays conducted with *Autographa californica* nucleopolyhedrovirus FV11 on selected lepidoteran hosts (*Spodoptera exigua*, *Helicoverpa armigera* and *Lymantria dispar*) confirm the host range corresponds to that of published studies on other *Autographa californica* nucleopolyhedrovirus isolates.

The host range of baculoviruses is restricted to terrestrial arthropods; primarily larval stages. In the class Insecta, only three orders are confirmed hosts of baculoviruses. All baculoviruses are restricted to order, and within that order, most are restricted to a single family and usually to a single species or only to few closely related species. Single Nucleopolyhedrovirus (SNPV) alphabaculoviruses and gammabaculoviruses (for example, *Autographa californica* nucleopolyhedrovirus and *Mamestra brassicae* nucleopolyhedrovirus (MabrNPV) can infect greater than 50 species crossing over 13 families of Lepidoptera. Only Lepidotera (Alphabaculovirus, Betabaculovirus), hymenopteran sawflies (Gammabaculovirus) and a few species of diptera (Deltabaculovirus) have been confirmed to host baculoviruses. There is no cross infection of baculoviruses between these orders. Baculoviruses do not infect cockroaches, grasshoppers, aphids, nor have they been shown to infect non-phytophagous beneficial and predatory insects such as lady beetles, parasitoids and honeybees. Although not infecting parasitoids, baculoviruses can cause premature death of larval host and competition for resources that can affect the fitness and survival of parasitoids. Parasitoids are often generalists and while a depletion of virally-treated insect populations will occur, the lack of non-target effects on other potential hosts would likely provide alternate hosts for the parasitoids. In addition studies suggest that some parasitoids transmit baculoviruses (for example, *Lymantria dispar* nucleopolyhedrovirus) and contribute to viral epizootics.

Autographa californica nucleopolyhedrovirus has been recognized as having a relatively wide host range amongst baculoviruses. From an extensive review of the literature, there were 59 species from 13 families listed as permissive host of *Autographa californica* nucleopolyhedrovirus. However, these results were limited, as few of the studies provided actual LD_{50} values and in some studies latent infections could not be ruled out. Furthermore some of the reported permissive hosts required high doses (not biologically relevant) to produce low levels of mortality and very few studies have assessed the production capacity of *Autographa californica* nucleopolyhedrovirus in alternate hosts. Hence for many species, the potential for propagation within a given non-target population has not been demonstrated.

In order to compare the host range similarity of *Autographa californica* nucleopolyhedrovirus FV11 to that of other *Autographa californica* nucleopolyhedrovirus strains that have been assessed in the literature, *Autographa californica* nucleopolyhedrovirus FV11 was tested against its natural host the cabbage looper (*Trichoplusia ni*), one permissive host, *Spodoptera exigua*, as well as two non-permissive hosts, *Helicoverpa armigera* and *Lymantria dispar*. Larvae were allowed to feed on insect diet that was either surface contaminated or mixed in with different concentrations of *Autographa californica* nucleopolyhedrovirus FV11 occlusions bodies. To directly compare bioassays with the published literature, all larvae were allowed to feed on the contaminated diet for the duration of the test except for *L. dispar* larvae which was transferred onto uninoculated diet after 48 hours. The larvae were allowed to feed until the first larvae reached the last instar which was between nine and nineteen days post infection depending on species, at which time mortality rates were recorded. Doses ranged from 2×10^3 to 2×10^8 OBs/mL for *L. dispar*, 2×10^4 to 2×10^8 OBs/g for *H. armigera*, 5×10^2 to 6×10^4 OBs/g for *S. exigua* and 60 to 4×10^4 OBs/g for *T. ni*. Data from three replicates, each containing either 32 (*L. dispar* and *T. ni*) or 50 larvae (*S. exigua* and *H. armigera* per treatment were pooled and analyzed by probit analysis. The infectivity of *Autographa californica* nucleopolyhedrovirus FV11 in all species was lower than its natural host , *T.ni* where potency ratios ranged from between as little as 114 times less infectious in permissive host (*S. exigua)* to as much as 286 to 207 times less infectious in the non-permissive host (*L. dispar*). All species used gave LD_{50} values that were comparable or higher (less infectious) than previously published assays with other *Autographa californica* nucleopolyhedrovirus strains, with the exception of *T. ni* which was a slightly more permissive host (64×) to *Autographa californica* nucleopolyhedrovirus FV11. Therefore, *Autographa californica* nucleopolyhedrovirus FV11 has a host range and virulence similar to that of other *Autographa californica* nucleopolyhedrovirus strains.

An independent search of published scientific literature through PubMed yielded no reports of adverse effects to birds, plants, wild mammals, arthropods (with the exception of known hosts) and non-arthropod invertebrates.

Based on all the available information on the biological properties of *Autographa californica* nucleopolyhedrovirus FV11 and its anticipated effects on non-target terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, terrestrial non-target arthropod invertebrates, non-arthropod invertebrates, and terrestrial plants from the proposed use of Loopex in greenhouses.

4.2.2 Effects on Aquatic Organisms

Acceptable scientific rationales were submitted to waive Tier I testing requirements for aqautic non-target organisms based on an extensive review of the published scientific literature including the results of ecotoxicological testing conducted on various baculoviruses. The rationales were based on the following:

- baculoviruses are not toxic to aquatic vertebrate animals (fish), arthropods, non-arthropod invertebrates, and plants, supported by a lack of adverse effects to these non-target organisms reported in the scientific literature;
- baculoviruses are infectious only to insects of the same order from which they were initially isolated; and
- baculoviruses are ubiquitous and persistent in aquatic ecosystems yet there has been no report of negative impact of baculoviruses on ecosystems other than the effect on the target host insect.

Fish

In a 30-day study, 2 groups (36/group) of rainbow trout (*Oncorhynchus mykiss)* were exposed aquatically (2.4×10^4 virus per L of water) and orally (intubation) at a dose of 3×10^6 *Neodiprion lecontei* polyhedrosis virus (NeleNPV) per gram of fish. Following treatment, six fish from each group were sacrificed on Days 0, 1, 3, 7, 14 and 30. At the time of necropsy, serum samples, tissues sample for histology and frozen samples for virus inoculation studies were taken. No mortalities occurred and no adverse effects were reported for the study. Rainbow trout were not affected by aquatic or dietary exposure of *Neodiprion lecontei* polyhedrosis virus (NeleNPV).

In another study, young (fingerling stage) salmonid species (*Oncorhynchus tshawytscha*, *O. kistustch* and *O. mykiss*) were exposed by three routes (intraperitoneal injection or IP, in the diet and aquatically). The three species of fish were exposed by the IP route to 1.7×10^2 OpMNV budded virions per fish and observed for adverse effects for 30 days following injection. Fish of all three species were exposed aquatically (18 hours) and in the diet (24 hours) to OpMNV at a 100-acre equivalent dose. After the exposure period, fish were transferred to non-inoculated water and observed for 30 days. Fish were randomly selected, sacrificed and then subjected to gross and histological examinations of tissues. In order to determine if OpMNV persisted in fish, *O. kistustch* were treated as above by IP, dietary and aquatic exposure. Three fish were sacrificed at each sampling time (0, 12, 48 and 96 hours post treatment) and organ tissues were pooled, alongside intestine and processed and bioassayed in *Orgyia pseudotsugata* larvae. OpMNV did not cause any pathological changes in any fish species by any routes of exposure. Bioassay results indicated the virus was cleared or inactivated within 8 hours for aquatic exposure or 24 hours for IP and dietary exposure. None of the salmonid species showed pathology when exposed to OpMNV by the three different routes. Both polyhedral and non-occluded virions were inactivated by *O. kistustch* exposed to the virus by three different routes.

Arthropods

In a 30-day dietary study, 120 grass shrimp (*Palaemonetes vulgaris*) (two per container) were fed twice weekly pellets containing 1.5×10^7 PIBs of *Autographa californica*/pellet. There were no significant differences in mortality between treated and control groups. There were no adverse effects on feeding behaviour, equilibrium or activity. Histopathological, ultrastructural and serological results indicated that the dietary exposure did not result in infection or related pathogenicity.

In a 21-day chronic toxicity study, *Daphnia magna* were exposed aquatically under controlled conditions to 10^2, 10^3, 10^4, 10^5, 10^6 *Neodiprion abieitis* Nucleopolyhedrovirus (NeabNPV) OBs/mL. Adult survival was 95% to 10% among treatment groups. Mean number of neonates per surviving parent animal was 98 to 114. There were no significant differences in adult survival or mean number of neonates per surviving parent animal $P>0.05$. For all endpoints assessed in the study, the no effect concentration was 10^6 OBs/mL and the lowest effect concentration was greater than 10^6 OBs/mL. Due to a lack of concentration response, the median effective concentration (EC_{50}) for reproductive output was estimated to be greater than 10^6 OBs/mL.

An independent search of published scientific literature through PubMed yielded no reports of adverse effects to fish, aquatic arthropods and non-arthropod invertebrates, and aquatic plants.

Based on all the available information on the effects of *Autographa californica* nucleopolyhedrovirus virus FV11 to non-target aquatic organisms there is reasonable certainty that no harm will be caused to fish, aquatic arthropod and non-arthropod invertebrates, and aquatic plants from the proposed use of Loopex in greenhouses. As a general precaution, the label will prohibit the direct application of Loopex to aquatic habitats, estuaries or marine habitats, and direct handlers to not contaminate surface water by disposal of equipment wash waters.

4.3 Incident Reports related to the Environment

Since 26 April 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the PMRA website. Incidents were reviewed for *Autographa californica* nucleopolyhedrovirus FV11 as well as other registered baculoviruses (Douglas fir tussock moth nucleopolyhedrovirus, NeleNPV, LdNPV, CpGV strains CMGV4 and M, and NeabNPV). As of 22 August 2014, no incident reports involving these baculoviruses have been reported to the PMRA.

5.0 Value

5.1 Effectiveness Against Pests

Five greenhouse efficacy trials were submitted in support of this application. The efficacy data demonstrate that the product, at rates of 2.5×10^{10}- 1×10^{11} PIB/ha applied in a spray volume of 400 L/ha, provides control of cabbage looper larvae on the target crops. Reduction in leaf and fruit damage was also observed, although not to a significant degree. The data also demonstrate that the virus does not lose its effectiveness until 7-14 days after application.

5.2 Non-Safety Adverse Effects

No phytotoxicity was observed in any of the reviewed efficacy trials.

5.3 Consideration of Benefits

Autographa californica nucleopolyhedrovirus virus is listed as an intermediate priority in the Canadian Grower Priority Database for control of cabbage looper on greenhouse tomato, cucumber and pepper. Registered alternatives for control of cabbage looper on greenhouse tomato, cucumber and pepper are *Bacillus thuringiensis* (Bt), spinosad, chlorantraniliprole, tebufenozide (greenhouse tomato and pepper only) and chlorfenapyr (greenhouse tomato and pepper only). Due to its narrow host spectrum (it infects only some species from the Noctuidae family of Lepidoptera), *Autographa californica* nucleopolyhedrovirus virus is compatible with the use of beneficial insects for biological control. It can be used in rotation with Bt or chemical insecticides.

Resistance to *Autographa californica* nucleopolyhedrovirus virus in cabbage looper has not been reported. *Autographa californica* nucleopolyhedrovirus virus represents a new mode of action for use against cabbage looper larvae. Based on the demonstrated efficacy of the product and its compatibility with Bt, chemical insecticides and beneficial insect species, Loopex could be a valuable part of an integrated pest management program on greenhouse tomato, cucumber and pepper.

5.4 Supported Uses

Use of Loopex for control of cabbage looper larvae on greenhouse tomato, cucumber and pepper is supported.

6.0 Pest Control Product Policy Considerations

6.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, i.e. persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*.

AcMNPV Technical and Loopex were assessed in accordance with the PMRA Regulatory Directive DIR99-03.[5]

- AcMNPV Technical does not meet the Track 1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.
- There are also no formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria.

6.2 Formulants and Contaminants of Health Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[8] and taking into consideration the Ozone-depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

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5 Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*.

6 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part 1 Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

7 Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act.*

8 Regulatory Directive DIR2006-02, *Formulants Policy and Implementation Guidance Document.*

- The technical grade active ingredient, AcMNPV Technical, does not contain formulants of health or environmental concern as identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.
- The end-use product, Loopex, does not contain formulants of health or environmental concern as identified in the *Canada Gazette*, Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006–02.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for AcMNPV Technical and Loopex were judged to be adequate to assess their potential human health and environmental risks. The technical product was characterized and the specifications of the end-use product were supported by the analyses of a sufficient number of batches. Storage stability data are required. In the interim, the labels for AcMNPV Technical and Loopex must indicate a maximum storage period of six months at 4°C or less.

7.2 Human Health and Safety

The scientific waiver rationales and acute toxicity and infectivity studies using other baculoviruses submitted in support of *Autographa californica* nucleopolyhedrovirus FV11 were determined to be sufficiently complete to permit a decision on registration. *Autographa californica* nucleopolyhedrovirus is expected to be of low toxicity and not infective or pathogenic by the oral, pulmonary, intravenous and dermal routes of exposure. The information also suggests that Loopex will not be irritating to the skin. Loopex is considered an eye irritant due to the absence of the eye irritation study and, therefore, the signal words "CAUTION – EYE IRRITANT" must appear on the principal display panel of the label. Since AcMNPV Technical and Loopex are considered potential sensitizers, the signal words, "POTENTIAL SENSITIZER", are also required on the principal display panel of both products.

When handled according to prescribed label instructions, the potential for dermal, eye and inhalation exposure for mixer/loaders, applicators, and handlers exists, with the primary source of exposure to workers being dermal.

In individuals exposed to large quantities of Loopex, respiratory and dermal sensitivity could possibly develop upon repeated exposure to the product since all microorganisms, including *Autographa californica* nucleopolyhedrovirus virus FV11, contain substances that are potential sensitizers. Therefore, anyone handling or applying Loopex must wear a long-sleeved shirt, long pants, shoes plus socks, waterproof gloves, eye goggles and a NIOSH approved mist filtering mask or respirator with any N-95, P-95 or R-95 filter. In addition, all unprotected workers are restricted from entering enclosed areas (including greenhouses) where Loopex has been applied until mists have settled.

The health risk to the general population, including infants and children, as a result of bystander exposure and/or chronic dietary exposure is expected to be negligible and of no concern. This based on the facts the proposed use is in greenhouses, the low toxicity/pathogenicity profile for *Autographa californica* nucleopolyhedrovirus FV11, AcMNPV Technical and Loopex and the absence of sustained increases in exposure to bystanders beyond natural levels. The specification of an MRL under the *Pest Control Products Act* is not required for *Autographa californica* nucleopolyhedrovirus virus FV11.

7.3 Environmental Risk

The scientific rationales and supporting published scientific literature submitted in support of AcMNPV Technical and Loopex were determined to be sufficiently complete to permit a decision on registration. The greenhouse use of Loopex containing *Autographa californica* nucleopolyhedrovirus FV11 is not expected to pose a risk to non-target organisms when the directions for use on the label are followed.

Based on the proposed greenhouse use for Loopex, exposure to terrestrial and aquatic environments is expected to be minimal. As a general precaution, however, the product label will prohibit the direct application of Loopex to aquatic habitats (such as lakes, rivers, sloughs, ponds, prairie potholes, creeks, marshes, streams, reservoirs, and wetlands), estuaries or marine habitats, and direct handlers to not contaminate surface water by disposal of equipment wash waters.

7.4 Value

Loopex has value for control of cabbage looper larvae on greenhouse tomato, cucumber and pepper. The supported uses address priorities from the Canadian Grower Priority Database, and *Autographa californica* nucleopolyhedrovirus contributes to resistance management because it is a new mode of action for use against cabbage looper. *Autographa californica* nucleopolyhedrovirus is compatible with the use of beneficial insects for biological control, and it can be used in rotation with Bt or chemical insecticides. Therefore, it could be a valuable part of an integrated pest management program on greenhouse tomato, cucumber and pepper.

8.0 Proposed Regulatory Decision

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of AcMNPV Technical and Loopex, containing the technical grade active ingredient *Autographa californica* nucleopolyhedrovirus FV11, to control cabbage looper larvae in greenhouse cucumber, peppers and tomatoes.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations and Acronyms

°C	degree(s) Celsius
AcMNPV	*Autographa californica* (multiple) nucleopolyhedrovirus
AcNPV	*Autographa californica* nucleopolyhedrovirus
BSL-1	BioSafety Level 1
Bv	budded virus
Bw	body weight
Cf	*Choristoneura fumiferana*
CfNPV	*Choristoneura fumiferana* nucleopolyhedrovirus
Cm	Centimetres
CpGV	*Cydia pomonella* granulovirus
cSt	Centistoke
DNA	deoxyribonucleic acid
DNCB	Dinitrochlorobenzene
EC_{50}	median effect concentration
EP	end use product
FHSA	Federal Hazardous Substances Act
FV	Fraser Valley
g	gram
Kg	Kilogram
L	litre
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LdMNPV	*Lymantria dispar* nucleopolyhedrovirus
NeleNPV	*Neopridion lecontei* nucleopolyhedrovirus
mL	millilitre
MPCA	microbial pest control agent
MRL	maximum residue limit
NeabNPV	Neodiprion abietis nucleopolyhedrovirus
NeleNPV	Neodiprion lecontei nucleopolyhedrovirus
NIOSH	National Institute for Occupational Safety and Health
NPV	nucleopolyhedrovirus
OBs	Occlusion bodies
ODV	occlusion derived virus
OpMNV	*Orygia pseudotsugata* nucleopolyhedrovirus
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PIBs	polyhedral inclusion bodies
PMRA	Pest Management Regulatory Agency
REN	restriction endonuclease
RNA	ribonucleic acid
SlNPV	Spodopera littoralis nucleopolyhedrovirus
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy

Appendix I

Table 1 Toxicity and Infectivity of AcMNPV Technical and Loopex

Study Type	Species, Strain, and Doses	Results	Comments	Reference(s)
Acute Toxicity/Infectivity of AcMNPV Technical				
Acute Oral Toxicity, Acute Pulmonary Toxicity, Intravenous Infectivity	A scientific rationale was submitted by the applicant to waive the requirement for acute oral toxicity, acute pulmonary toxicity, and intravenous infectivity based on the lack of reported adverse effects despite the natural occurrence and prevalence of baculoviruses in the environment, the limited host range associated with baculoviruses, the absence of virus replication or expression in non-permissive cells and the results of numerous safety studies conducted with baculoviruses on vertebrates and mammals. The request to waive testing was accepted.			2329598 2329602* 2329613* 2329619* 2329622* 2329623* 2329624* 2329626* 2329628* 2329644* 2329646* 2329671* 2329676* 2329680* 2329696* 2329699* 2329700* 2329701* 2329702* 2329706* 2329707* 2329710* 2329716* 2329720* 2329723 2329725 2329726 2329727 2329736 2329744 2329726 2410352*

Tissue Culture	A scientific rationale was submitted by the applicant to waive the requirement for tissue culture testing based on the lack of reported adverse effects despite the natural occurrence and prevalence of baculoviruses in the environment, the limited host range associated with baculoviruses, the absence of virus replication or expression in non-permissive cells and the results of numerous safety studies conducted with baculoviruses on vertebrates and mammals. The request to waive testing was accepted.	References above marked with * and 2329760 2329761 2329762 2329764
Acute Toxicity and Irritation of Loopex		
Acute Dermal Toxicity	A scientific rationale was submitted by the applicant to waive the requirement for acute dermal toxicity testing based on the lack of reported adverse effects despite the natural occurrence and prevalence of baculoviruses in the environment, the limited host range associated with baculoviruses, the absence of virus replication or expression in non-permissive cells, the results of numerous dermal studies conducted with baculoviruses on laboratory animals and the identity and prevalent use of the formulation ingredients in consumer products. The request to waive testing was accepted.	References above marked with * and 2329750 2329752 2410353
Dermal Irritation	A scientific rationale was submitted by the applicant to waive the requirement for dermal irritation testing based on the lack of reported adverse effects despite the natural occurrence and prevalence of baculoviruses in the environment, the limited host range associated with baculoviruses, the absence of virus replication or expression in non-permissive cells, the results of numerous dermal irritation studies conducted with baculoviruses on laboratory animals and the identity and prevalent use of the formulation ingredients in consumer products. The request to waive testing was accepted.	References above marked with * and 2329754 2329755 2329756 2410353 2410358
Eye Irritation	No information was submitted to address eye irritation.	-
Sensitization	A sensitization study indicated that *Neodiprion lecontei* NPV does not cause a delayed skin reaction. The PMRA assumes, however, that all microorganisms contain substances that can elicit positive hypersensitivity reactions regardless of the outcome of sensitization testing.	2329758 2329759

Table 2: Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds	A request to waive the requirement for test data was submitted based on the host specificity/narrow host range of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to birds.			PMRA 2329768 2329783
Wild Mammals	A request to waive the requirement for test data was submitted based on the host specificity/narrow host range of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to non-target wild mammals.			PMRA 2329768 2329786
Invertebrates				
Arthropods				
Terrestrial Arthropods	A request to waive the requirement for test data was submitted based on the host specificity/narrow host range of the MPCA and results of bioassays that confirm AcMNPVFV11 has a similar host range to other AcMNPV isolates and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to terrestrial arthropods.			PMRA 2329768 2329806
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A request to waive the requirement for test data was submitted based on the host specificity/narrow host range of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to non-target terrestrial non-arthropod invertebrates.			PMRA 2329768 2329811 2329791
Plants				
Plants	A request to waive the requirement for test data was submitted. Baculoviruses only infect insect larvae and there is a lack of reports of negative effects on plants in published scientific literature. No further data are required to address the hazard to terrestrial plants.			PMRA 2329768 2329812
Microorganisms				
Micro-organisms	A request to waive the requirement for test data was submitted. Baculoviruses only infect insect larvae and there is a lack of reports of negative effects on microorganism. No further data are required to assess the risk of harm to microorganisms.			PMRA 2329768

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Aquatic Organisms				
		Vertebrates		
Fish	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to fish.			PMRA 2329768 239788 239789 239790
		Invertebrates		
Aquatic Arthropods	A request to waive the requirement for test data was submitted based on the host specificity/narrow host range of the MPCA and that no reports of adverse effects were found in published scientific literature . No further data are required to assess the risk of harm aquatic arthropods.			PMRA 2329768 2329808
Aquatic Non-Arthropod Invertebrates	A request to waive the requirement for test data was submitted based on the host specificity/narrow host range of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to aquatic non-arthropod invertebrates.			PMRA 2329768
		Plants		
Aquatic Plants	A request to waive the requirement for test data was submitted based on the host specificity of the MPCA and that no reports of adverse effects were found in published scientific literature. No further data are required to assess the risk of harm to aquatic plants.			PMRA 2329813 2329768

References

PMRA Document Number	Reference
2329602	2003, ENVIRONMENTAL IMPACTS OF MICROBIAL INSECTICIDES, DACO: M1.2,M2.7.2,M4.1,M9.1,M9.5.1
2329612	2003, Can Host Susceptibility to Baculovirus Infection be Predicted from Host Taxonomy or Life History?, DACO: M1.2,M2.7.2,M9.5.1
2329613	BIOLOGY OF BACULOVIRUSES VOLUME 1 BIOLOGICAL PROPERTIES AND MOLECULAR BIOLOGY, DACO: M1.2,M2.7.2,M4.1,M9.5.1
2329614	1997, Liquefaction of Autographa californica Nucleopolyhedrovirus-Infected Insects Is Dependent on the Integrity of Virus-Encoded Chitinase and Cathepsin Genes, DACO: M1.2,M2.7.2
2329616	2001, Autographa californica M Nucleopolyhedrovirus ProV-CATH is Activated during Infected Cell Death, DACO: M1.2,M2.7.2
2329617	1991, A NEW BROAD HOST SPECTRUM NUCLEAR POLYHEDROSIS VIRUS ISOLATED FROM A CELERY LOOPER,ANAGRAPHA FALCIFERA, DACO: M1.2,M2.7.2,M9.5.1
2329622	Recent Advances in Our Knowledge of Baculovirus Molecular Biology and Its Relevance for the Registration of Baculovirus-Based Products for Insect Pest Population Control, DACO: M1.2,M2.7.2,M4.1,M9.1,M9.5.1
2329624	2002, ENVIRONMENT DIRECTORATE JOINT MEETING OF THE CHEMICALS AND THE WORKING PARTY ON CHEMICALS,PESTICIDES AND BIOCHNOLOGY, DACO: M1.2,M2.7.2,M4.1,M9.1
2329625	1986, INSECT PATHOGENIC VIRUSES AS PEST CONTROL AGENTS, DACO: M1.2,M2.7.2,M9.5.1
2329626	2011, BACULOVIRUS MOLECULAR BIOLOGY, DACO: M1.2,M2.7.2,M4.1,M9.5.1
2329628	2007, THE BACULOVIRUSES OCCLUSION-DERIVED VIRUS: VIRION STRUCTURE AND FUNCTION, DACO: M1.2,M10.3.2.2,M2.7.2,M4.1
2329631	2009, Baculovirus Host-Range, DACO: M1.2,M2.7.2,M4.1,M9.5.1
2329632	1973, INFECTIVITY OF A NUCLEAR POLYHEDROSIS VIRUS FROM THE ALFLFA LOOPER, DACO: M1.2,M2.7.2,M9.5.1
2329641	M 2.7 Characterization of the MPCA, DACO: M2.7.1

2329643	M2 Product Characterization and Analysis M2.7 Characterization of MPCA M 2.7.2 Biological Properties of the MPCA., DACO: M2.7.2
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